Study on the Growth Law of Photosynthetic Bacteria in Wastewater Treatment

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Abstract: There are many methods to measure microbial growth. In this study, we took photosynthetic bacteria (PSB) as the main body and optimized its culture medium formula. Our study displayed that the optimal medium formula for PSB was sodium acetate 3.3 g/L, NH4Cl 0.6 g/L, KH2PO4 0.9 g/L, MgSO4 0.5 g/L, and beef extract 1.5 g/L. The optimized medium could effectively facilitate the growth of PSB.

1 INTRODUCTION

1.1 Photosynthetic Bacteria

Photosynthetic Bacteria (PSB), a type of bacteria that can produce photosynthesis, are one of the earliest bacteria on earth. They utilize various amino acids, sugars, fatty acids or ethanol in the environment as hydrogen donors to synthesize organic compounds under photosynthesis. They can adapt to different ecological environments and are mainly distributed in the soil and the silt of rivers, ponds, sewage ditches, oxidation ponds, and oceans. PSB are widely distributed and have many kinds. According to whether they generate oxygen during photosynthesis, they can be divided into oxygen-producing PSB represented by cyanobacteria and non-oxygen-producing PSB represented by rhodopseudomonas. Generally, non-oxygen-producing PSB include four groups of green non-sulfur bacteria, purple sulfur bacteria, green sulfur bacteria, and purple non-sulfur bacteria. Oxygen-producing PSB, usually called aerobic photosynthetic bacteria, are divided into cyanobacteria and prochlorophyte.

PSB wastewater treatment technology is a newly emerging wastewater treatment method in recent years. Compared with the conventional activated sludge wastewater treatment technology, it has the advantages of recovering single-cell protein and no secondary pollutants, hence it has attracted wide attention. The PSB wastewater treatment technology can recycle and reuse biological resources while reducing pollutants and protecting the environment, so as to achieve the unity of environmental and economic benefits. Wastewater contains a lot of organic solids, the water body is dark brown, and it can cause irreversible pollution to groundwater. PSB have a variety of metabolic pathways and can efficiently treat organic wastewater. Their unique light-energy heterotrophic characteristics enable them to survive in high-concentration organic wastewater, and finally convert the organic matter in the wastewater into nutrients needed for the growth of aquatic organisms through metabolism to achieve the purpose of purifying water quality. PSB have strong vitality and are widely distributed, playing a vital role in wastewater treatment, biological feed, and biological hydrogen refining. Nevertheless, due to the low biomass and high cost of cultivation during the cultivation process, the application prospects of PSB are restricted.

2 METHODS TO MEASURE THE GROWTH OF PSB

Methods to determine the number of PSB contain direct microscope counting, dilution plate counting, dilution culture counting, and turbidimetry.

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2.1 Direct Microscope Counting Method

Direct microscope counting method is a quick and convenient method that place an appropriate amount of microbial sample suspension to be tested on a special counting plate with a fixed area and volume, and then count the microorganisms directly under the microscope. At present, there are mainly two types of counting plates in the laboratory. Bacterial counters can be utilized for general bacteria while hemocytometers can be used for larger yeast or mold spores. The principles and methods of use of these two types of counting plates are the same, except that the thinner bacteria counting plates are better for observation with oil mirrors.

2.2 Dilute Plate Counting Method

The dilute plate counting method is a method of diluting the tested sample solution by an appropriate multiple to which the microorganisms are dispersed into single cells, and then measuring the number of microorganisms by the number of single colonies formed on the solid medium under suitable growth conditions. In actual operation, firstly, the sample solution needs to be diluted gradually. Then, a certain amount of sample solution is uniformly spread on the solid medium with appropriate growth conditions and cultivated upside down for a certain time. Finally, the colonies on the plate are counted. The most significant step of this method is dilution. Choosing an appropriate dilution factor can decrease errors and improve the accuracy of the determination.

2.3 Dilution Culture Counting Method

The dilution culture counting method, also known as the maximum probability method, is based on mathematical probability and statistics. In this method, a series of dilutions of the bacterial culture solution are carried out until the diluted solution is inoculated on the culture medium and no or very little bacterial growth occurs. Based on the lowest dilution at which growth occurs and the highest dilution at which no growth occurs, the method relies on the "Most probable number (MPN)" theory to calculate the approximate number of bacteria per unit volume of the sample.

3 MATERIALS AND METHODS

3.1 Experimental Strains

PSB obtained by separation and culture in the laboratory of our college.

3.2 Medium

3.2.1 Liquid Culture Medium

2.5 g of sodium acetate, 2.0 g of beef extract, 0.5 g of $MgSO_4$, 1.0 g of NH_4Cl, 0.5 g of KH_2PO_4 and 1000 mL of sterile water.

3.2.2 Solid Medium

1.5 g of beef extract, 5.0 g of peptone, 2.5 g of NaCl, 7.0 g of agar and 500 mL of sterile water.

3.3 Experimental Equipment

MGC-300 light incubator, 752 UV-Vis spectrophotometer and YXQ-50S11 high-pressure steam sterilization pot.

3.4 Determination of The Standard Curve of PSB

3.4.1 Determination of Optical Density (OD_{660})

The bacterial concentration of PSB was expressed by optical density (OD_{660}). After the bacteria solution was appropriately diluted, the absorbance value was measured at a wavelength of 660 nm, with a blank liquid medium used as a control.

The optical density value of the bacterial cell (OD660) = OD value × dilution multiple, and the regression equation measured was as follows:

$$y=0.50601x \ (r=0.99907) \quad (1)$$

In this equation, x represents the concentration of PSB, y represents the OD_{660} value, and r represents the correlation coefficient.

3.4.2 Dilute Plate Counting Experiment

Nine sets of sterile petri dishes were taken, and 3 sets of 10^{-5},10^{-6}, and 10^{-7} were marked with markers. The PSB suspension was diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} in a 10-fold concentration gradient. Then, 3 1 mL sterile straws were utilized to draw 0.1 mL of 10^{-5}, 10^{-6} and 10^{-7} diluted bacterial suspension which were put them into numbered sterile petri

dishes. The petri dish was placed in a constant temperature medium at 32 °C for cultivation. After the bacterial suspension was cultured for 48 h, the petri dish was taken out. Subsequently, the colonies formed in each petri dish were counted, and the average number of colonies on the 3 plates of the same dilution was calculated according to the following formula:

Colony-forming unit per milliliter (CFU) = average number of colonies × dilution factor × 10.

3.4.3 Orthogonal Experiment

PSB can directly treat high-concentration organic wastewater without the problem of sludge treatment. For the moment, there are many studies on the optimization of the growth conditions of PSB. These conditions play a crucial part in the growth and reproduction of PSB and have very paramount practical significance. The growth of PSB is affected by many factors. The experiment is designed according to the $L_{16}(45)$ orthogonal experiment table, and the best medium for PSB is screened out.

Table 1: Orthogonal experiment factor level table of PSB culture medium.

Level	A	B	C	D	E
	Sodium acetate (g/L)	NH_4Cl (g/L)	KH_2PO_4 (g/L)	$MgSO_4$ (g/L)	Beef extract (g/L)
1	2.1	0.6	0.3	0.3	0.5
2	2.5	1.0	0.5	0.5	1.0
3	2.9	1.4	0.7	0.7	1.5
4	3.3	1.8	0.9	0.9	2.0

4 EXPERIMENTAL RESULTS

4.1 Dilute Plate Counting Results (Table 2).

Table 2 shows the number of photosynthetic bacteria and the results of plate colony counting.

Table 2: Dilute plate counting results.

Dilatability	10^{-5}				10^{-6}				10^{-7}			
	1	2	3	Average	1	2	3	Average	1	2	3	Average
CFU/Plate	2182	1916	1963	2020	267	281	226	258	31	42	33	35
CFU/mL	2.02×10^9				2.58×10^9				3.53×10^9			

4.2 Experimental Results of OD_{660} Value of PSB Suspension (Table 3)

To investigate the growth of photosynthetic bacteria, the experimental results of bacterial concentration OD_{660} of photosynthetic bacteria are shown in Table 3.

Table 3: OD_{660} value results of PSB suspension.

Sample	Concentration (g/L)	Mean concentration (g/L)	Average (Abs)	Standard deviation (SD)	Relative Standard Deviation%(RSD)	Graduation (Abs)
3 parallel samples	3.1					1.550
	3.1	3.1	1.5636	0.01174	0.7511	1.566
	3.1					1.573

4.3 Orthogonal Experiment Results

Table 4: Orthogonal experiment result table.

Experimental group	A	B	C	D	E	OD_{660}
1	1	1	1	1	1	1.1532

2	1	2	2	2	2	1.4331
3	1	3	3	3	3	1.5001
4	1	4	4	4	4	1.4435
5	2	1	2	3	4	1.3505
6	2	2	1	4	3	1.6746
7	2	3	4	1	2	1.5173
8	2	4	3	2	1	1.4378
9	3	1	3	4	2	1.3607
10	3	2	4	3	1	1.5455
11	3	3	1	2	4	1.4484
12	3	4	2	1	3	1.4779
13	4	1	4	2	3	1.8409
14	4	2	3	1	4	1.3539
15	4	3	2	4	1	1.5259
16	4	4	1	3	2	1.7233
k_1	1.3825	1.4263	1.4999	1.3756	1.4156	
k_2	1.4951	1.5018	1.4469	1.5401	1.5086	
K_3	1.4581	1.4979	1.4131	1.5299	1.6234	
K_4	1.6110	1.5206	1.5868	1.5012	1.3991	
R	0.2285	0.0943	0.1737	0.1645	0.2243	

According to the optimization experiment of the proliferation medium formula and from the visual analysis of Table 4, it could be concluded that the order of each factor affecting the OD_{660} value of PSB growth was A>E>C>D>B. The factor A sodium acetate had the most evident effect on the growth of PSB while the factor B ammonium chloride had the least effect on the growth of PSB. The best medium formula combination for PSB was $A_4B_4C_4D_3E_3$. The optimal growth condition of the orthogonal experiment was experimental group 13, namely sodium acetate 3.3 g, NH_4Cl l0.6 g, KH_2PO_4 0.9 g, $MgSO_4$ 0.5 g, beef extract 1.5 g.

5 RESEARCH PROGRESS OF PSB IN WASTEWATER TREATMENT

PSB have different physiological and biochemical functions like nitrogen fixation, carbon fixation, dehydrogenation, and sulfide oxidation, playing a crucial part in the natural carbon, nitrogen, and sulfur cycles. As a result, it has significant scientific research value. The wastewater treatment technology of PSB is established on the basis of ecological succession laws and principles. In the 1960s, Kobayash et al. unveiled the role and principle of PSB in the self-purification process of natural wastewater. In the 1970s, the application of PSB in the purification and treatment of organic wastewater and the research as a source of feed protein were carried out abroad extensively. At present, hydrogen has become an efficient clean fuel. In the process of various biological hydrogen production, the application of PSB to produce hydrogen under light is the current research hotspot. The PSB hydrogen production process can be combined with the organic wastewater treatment process. The utilization of small molecular organic acids to produce clean energy under light can degrade organic matter in wastewater while producing hydrogen, hence, it is considered the most environmentally friendly hydrogen production method. For instance, Turkarslan et al. employed rhodobacter sphaeroides to produce hydrogen by photosynthesis based on wastewater produced in dairy plants. At the beginning of the experiment, the bacteria could not grow, but after adding a proper amount of malate to the wastewater, the bacteria began to grow and produce hydrogen.

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